

# ***Boletopsis nothofagi* sp. nov. associated with *Nothofagus* in the Southern Hemisphere**

Jerry A. Cooper<sup>1</sup>, Patrick Leonard<sup>2</sup>

**1** Landcare Research, PO Box 40, Lincoln, 7640, New Zealand **2** Motueka, New Zealand

Corresponding author: Jerry A. Cooper (CooperJ@landcareresearch.co.nz)

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## **Abstract**

*Boletopsis nothofagi* **sp. nov.**, an ectomycorrhizal taxon is described from *Nothofagus* forests in New Zealand. A comparison of available molecular ITS sequences, and morphological data was carried out to confirm the novelty of the taxon. This is the first report of the genus in the Southern Hemisphere.

## **Key words**

Bankeraceae, *Boletopsis nothofagi*, *Nothofagus*, New Zealand

## **Introduction**

A collection of a *Boletopsis* species was made in 2009 during the annual foray of the Fungal Network of New Zealand (FUNNZ), at the Orongorongo Valley in the Rimutaka Forest Park east of Wellington, North Island, New Zealand. The material was initially thought to be sterile but subsequent examination showed a few spores with the characteristic thelephoroid morphology of *Boletopsis*. A subsequent collection in 2010 from South Island and re-collection of material at the North Island site provided more fertile material and is the basis for this description of a new species. This appears to be the first record of the genus in the Southern Hemisphere.

*Boletopsis* is a genus of ectomycorrhizal, stipitate, poroid fungi phylogenetically related to the hydroid (toothed) genera *Phellodon*, *Hydnellum*, *Bankera* and *Sarcodon* and placed in the Bankeraceae (Kirk et al. 2008). Four species are currently recognised in the subgenus *Boletopsis* (Stalpers 1993; Watling and Milne 2006); *B. leucomelaena*



(Pers.) Fayod, the type of the genus, *B. grisea* (Peck) Bond. & Sing., *B. smithii* K. Harrison and *B. perplexa* Watling & J. Milne. *Boletopsis leucomelaena* and *B. grisea* are widely distributed across North America, Europe and Asia, but are nowhere common (Gilbertson 1986). *Boletopsis leucomelaena* is usually associated with *Picea*, whereas *B. grisea* and *B. perplexa* are associated with *Pinus*, but there are records of these species associated with hardwoods. The ectomycorrhizal association of *B. smithii* is unknown. Recent molecular data supports the existence of additional taxa in North America (Watling and Milne 2008). *Boletopsis grisea* has also been reported from oak dominated cloud forest in Costa Rica and this represents the most southerly record of *Boletopsis* subgenus *Boletopsis* to date (Mata and Ryvarden 2007). Two other species, *B. atrata* Ryvarden and *B. subcitrina* Corner are pleuropodal, without inflated hyphae, and possess spores with small warts or spines. These species were segregated into *Boletopsis* subgenus *Boletopsina* (Stalpers, 1993). *Boletopsis subcitrina* subsequently formed the basis of a new genus *Corneroporus* (Hattori, 2001) and it seems likely this group is more distantly related to *B. leucomelaena* than the remaining four accepted species.

## Methods

DNA was extracted from dried herbarium material of the collection PDD96007 using REDExtract-N-Amp Plant PCR Kits (Sigma, USA). The tissue was ground in extraction buffer with a plastic pestle in the Eppendorf tube, then DNA extraction and PCR were carried out following the manufacturer's instructions. The extract was sequenced for the rRNA loci ITS1+5.8+ITS2 and LSU following the methods of (Johnston and Park 2005). Primers were ITS1F and ITS4 for the ITS region and LR0R and LR6 for LSU (Gardes and Bruns 1993). The chromatographs were assembled using Geneious (Drummond et al. 2011). Existing sequences were downloaded from GenBank for *Boletopsis* species, related New Zealand material and selected sequences of members of the Bankeraceae from other parts of the world (Table 1). Few LSU sequences are available for *Boletopsis*. This study focussed on ITS sequences resulting from an analysis of the relationships between *B. grisea*, *B. leucomelaena* and *B. perplexa* (Watling and Milne 2008).

Data exchange between applications was facilitated using Alter (Glez-Peña et al. 2010). Sequence alignment was carried using MAFFT (Katoh et al. 2002) within Geneious using the G-INS-i algorithm. Gblocks (Castersana 2000) was used to eliminate poorly aligned segments, with the number of contiguous conserved positions set to 8, the minimum block set to 10, and allowed gap positions to half, resulting in an alignment of 493 bases. The alignment was analysed by Jmodeltest (Posada 2008). A best-fit model of nucleotide substitution of GTR+G was proposed by jmodeltest. Phylogenetic analyses were performed MrBayes v3.2 (Huelsenbeck and Ronquist 2001) using the recommended model with two sets of four chains, one cold and three heated, with a chain temperature of 0.2. A sequence of *Piptoporus betulinus* was selected as an outgroup. All prior probabilities were left on default values. The model was run with a sampling fre-



**Table 1.** Sequences considered in the analysis.

GenBank Accession Numbers	Taxon	Collection	Country	Host	Notes
AF438567	<i>Boletopsis leucomelaena</i>		Korea	?	Without ITS2
DQ408766	<i>Boletopsis</i> sp.	Holden 150627	UK	<i>Pinus sylvestris</i>	= <i>B. perplexa</i> in Watling and Milne 2008
DQ408767	<i>Boletopsis</i> sp.	Wat. 28788	UK	<i>Pinus sylvestris</i>	= <i>B. perplexa</i> in Watling and Milne 2008, ≡Holden 077897
DQ408768	<i>Boletopsis grisea</i>	Kytovuori 94-1247	Finland	<i>Pinus sylvestris</i>	≡Rec227607 see Watling and Milne 2008
DQ408769	<i>Boletopsis grisea</i>	Niemela 6166	Finland	<i>Pinus sylvestris</i>	≡Rec227608 see Watling and Milne 2008
DQ408770	<i>Boletopsis grisea</i>	Palmen Aug01	Finland	<i>Pinus sylvestris</i>	Partial ITS1. ≡Rec227609 see Watling and Milne 2008, duplicated as EF457900 <i>B. sp.</i>
DQ408771	<i>Boletopsis leucomelaena</i>	Niemela 7749	Finland	<i>Picea</i> sp.	
DQ484064	<i>Boletopsis leucomelaena</i>	PBM 2678/ AFTOL 1527	USA		
EF457898	<i>Boletopsis</i> sp.	Rec227649	USA	?	
EF457899	<i>Boletopsis grisea</i>	Rec227658	USA	<i>Tsuga canadensis</i>	
EF457900	<i>Boletopsis</i> sp.	Rec227652	USA	?	Short sequence. Duplicate reference to Rec227609 as <i>B. grisea</i> see Watling and Milne 2008,
EF457901	<i>Boletopsis grisea</i>	Rec227653	USA	?	=Rec227652? see Watling and Milne 2008
EF457902	<i>Boletopsis grisea</i>	Rec227659	USA	<i>Pinus sylvestris</i>	=Rec227653? see Watling and Milne 2008
EF457903	<i>Boletopsis grisea</i>	Rec227656	USA	<i>Pinus contorta</i>	
EU622325	<i>Boletopsis</i> sp.	SL23	UK?	?	
FJ845401	<i>Boletopsis subsquamosa</i>	SMI350	USA?	?	= <i>B. grisea</i> see Watling and Milne 2008
AY569026	<i>Hydnellum cumulatum</i>	SEW 69	USA	<i>Tsuga</i>	
GU222291	<i>Phellodon sinclairii</i>	PDD89028	NZ	<i>Nothofagus</i>	
GU222318	<i>Phellodon</i> sp.	PDD89898	NZ	<i>Nothofagus fusca</i>	
GU222316	<i>Phellodon</i> sp.	PDD89880	NZ	<i>Nothofagus</i>	
GQ267479	<i>Pseudotomentella</i>	K98C35T239	NZ	<i>Pinus radiata</i>	
GQ267480	<i>Pseudotomentella tristis</i>	K91C38T363	NZ	<i>Pinus radiata</i>	



GenBank Accession Numbers	Taxon	Collection	Country	Host	Notes
FJ845438	<i>Sarcodon imbricatus</i>	SMI347	Canada	<i>Pinus?</i>	
GQ267491	<i>Tomentella</i> sp.	K80C37T323	NZ	<i>Pinus radiata</i>	
HQ533015	<i>Tomentellopsis</i> sp.	PDD95789	NZ	<i>Nothofagus solandri</i>	<i>T.</i> cf. <i>zygodesmoides</i> , with pale brown spores
JQ417193	<i>Boletopsis nothofagi</i>	PD96007	NZ	<i>Nothofagus fusca</i>	South Island, St Arnaud

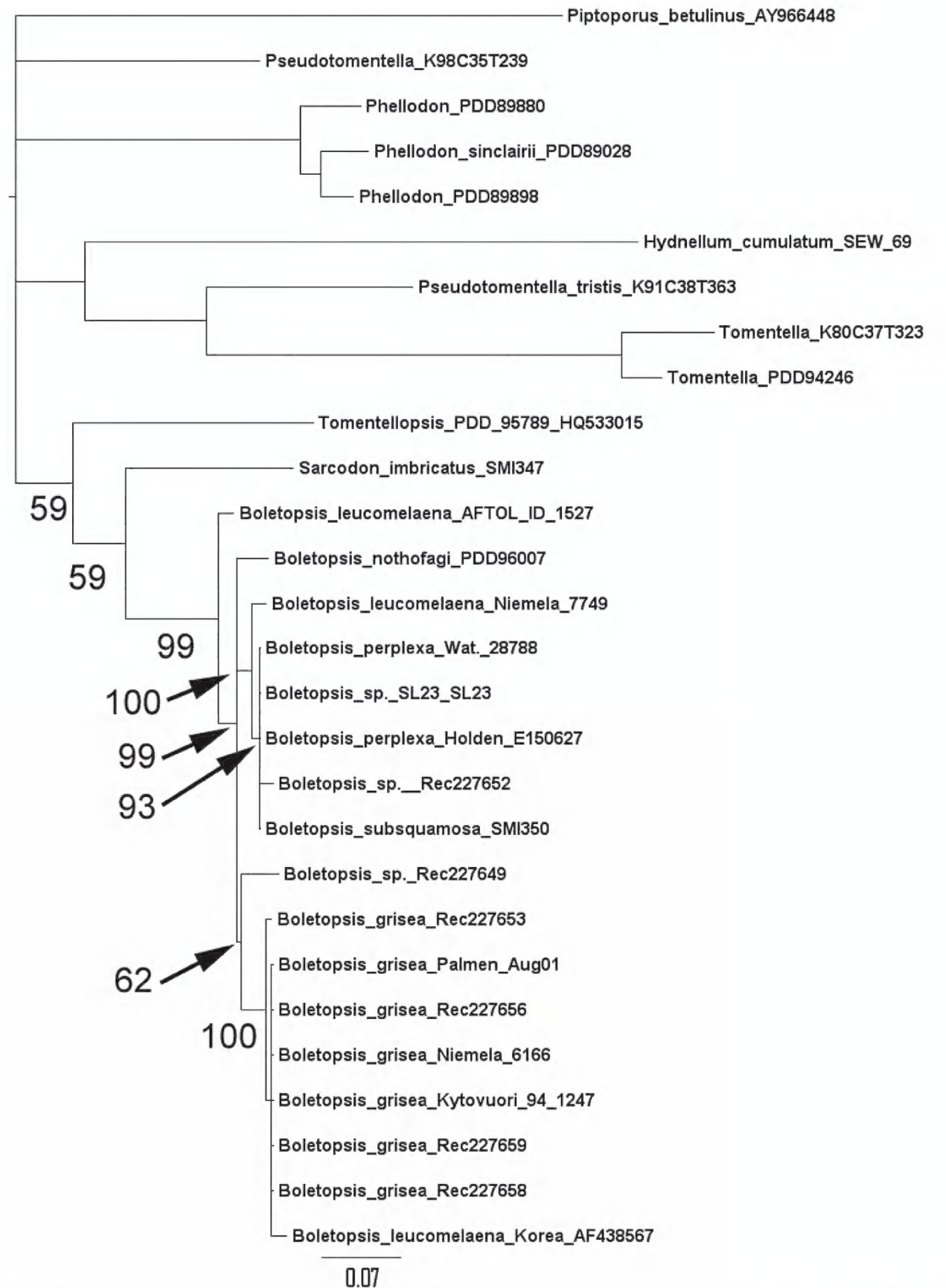
quency of 500 until the split-deviation frequency had fallen below 0.01, ca 1.2 million iterations. The results were examined to ensure good Metropolis coupling of chains and convergence statistics using Tracer (Rambaut and Drummond 2009). The first 25% of trees were removed in constructing a 50% majority rule consensus phylogram.

Results

Figure 1 shows the results of the phylogenetic analysis. The species concepts *B. grisea* and *B. perplexa* are supported. *Boletopsis* sp. (SL23), *B. subsquamosa* (SMI350), and *Boletopsis* sp. (Rec227652) are also referable to *B. perplexa*. The latter collections confirm the presence of this taxon in North America, as suggested by (Watling and Milne 2008). A consensus concept of *B. leucomelaena* is less well supported by these preliminary data with the collections appearing separately in the analysis. It is possible the current use of the name *B. leucomelaena* represents multiple cryptic taxa; it is reported as occurring in widely separate geographic regions and with differing ectomycorrhizal hosts. A similar situation was recently demonstrated in the case of European species of related *Hydnellum* and *Phellodon* (Ainsworth et al. 2010). *Boletopsis nothofagi* is clearly supported as a new taxon differing in 22 sites relative to *B. leucomelaena* (AFTOL, DQ484064) and 18 sites relative to *B. leucomelaena* (Niemela, DQ408771).

Discussion

The presence of *B. nothofagi* in New Zealand beech forests appears to have been overlooked despite a long history of the study of similar fungi in New Zealand (Cunningham 1958) (Maas Geesteranus 1971). This suggests *B. nothofagi* is a relatively rare (or rarely fruiting) indigenous member of the New Zealand ectomycorrhizal beech forest mycota. In addition no records of *Boletopsis* have been traced for any localities of naturally occurring *Nothofagus* forests in Australia, New Caledonia, New Guinea or South America. An alternative explanation for the presence of *B. nothofagi* in New Zealand is



**Figure 1.** Bayesian consensus phylogram, showing posterior probability, scale = substitutions per site

as a recent introduction of a species that usually has a different ectomycorrhizal host. Such introductions into New Zealand beech forests have occurred at least once in the case of *Amanita muscaria* (Johnston et al. 2008).



The absence of previous records of this recognisable species combined with the wide separation of the two currently known sites indicate that *Boletopsis nothofagi* is most likely a rare indigenous member of the New Zealand beech forest mycota. Its conservation status in New Zealand requires further investigation considering the status of other members of the family elsewhere in the world. For example many hydroid members of the family are threatened in Europe due to a variety of causes (Arnolds 2010). Many of these species are listed on European national red-data lists of fungi (Dahlberg and Mueller 2011). *Boletopsis grisea* is currently designated as threatened on five national lists and is subject to a number of management plans (Anon 1998-2011).

## Taxonomic treatment

***Boletopsis nothofagi* J.A. Cooper & P. Leonard, sp. nov.**

Registration Identifier: IndexFungorum IF550039

[http://species-id.net/wiki/Boletopsis\\_nothofagi](http://species-id.net/wiki/Boletopsis_nothofagi)

Holotype: PDD96007

**Description.** Basidiomes fasciculate, occasionally solitary, centrally stipitate, tough and fibrous. Pileus more or less convex, 10–80 mm diameter  $\times$  5–20 mm high, becoming undulate and edge somewhat incurved when young, smooth to finely fibrillose, grey [1D1, 1E1] (Kornerup and Wanscher 1989), weakly nigrescent when bruised and eventually becoming black. Stipe stuffed, clavate or cylindrical, 20–60 mm in length  $\times$  10–25 mm diameter, narrowing slightly at base and apex, smooth, dry, concolorous with pileus and darkening where bruised. Hymenial layer white 1–2 mm deep, bruising tan, pores angular, 2–3 per mm, drying pinkish tan, sometimes with lacerate edges. Pore layer extending slightly down the stipe and clearly delineated. Smell of dried material weakly of fenugreek, taste slightly acidic. Pileus immediately black in KOH, pigment leaching olivaceous black into white absorbent paper. Spore print not obtained.

Hyphal system monomitic. Pileus with a differentiated pileipellis consisting of a cutis, hyphae to 2  $\mu$ m diameter, with brown plasmonic pigment, hyphal surface covered in small amorphous granular material becoming dark green in KOH and dispersing into medium. Subcutis with inflated gloeoplerous-like hyphae, thin-walled, to 6  $\mu$ m diameter, clamped. Basidia pleurobasidial, cylindrical to clavate 5–10  $\times$  20–30  $\mu$ m, 4-spored, with basal clamp. Pores with fringe of slender clavate cystidia-like elements to 80  $\times$  4  $\mu$ m. Spores very pale tan, thin-walled, not dextrinoid or amyloid, non-cyanophilous, flat-topped tuberculate, with a narrowed waist. Spores dimensions are of a bounding rectangle encompassing maximum length and width of each spore. Length  $\mu$ =5.3  $\mu$ m,  $\sigma$ =0.5, width  $\mu$ =4.1  $\mu$ m,  $\sigma$ =0.5, Q  $\mu$ =1.35,  $\sigma$ =0.2 (combined statistics of measurement of 4, 13, 26, 20 spores from three fruiting bodies of the three collections).

**Distribution.** North and South Islands of New Zealand

**Ecology.** ectomycorrhizal in southern beech (*Nothofagus*) forests and so far found only in association with *Nothofagus fusca*.



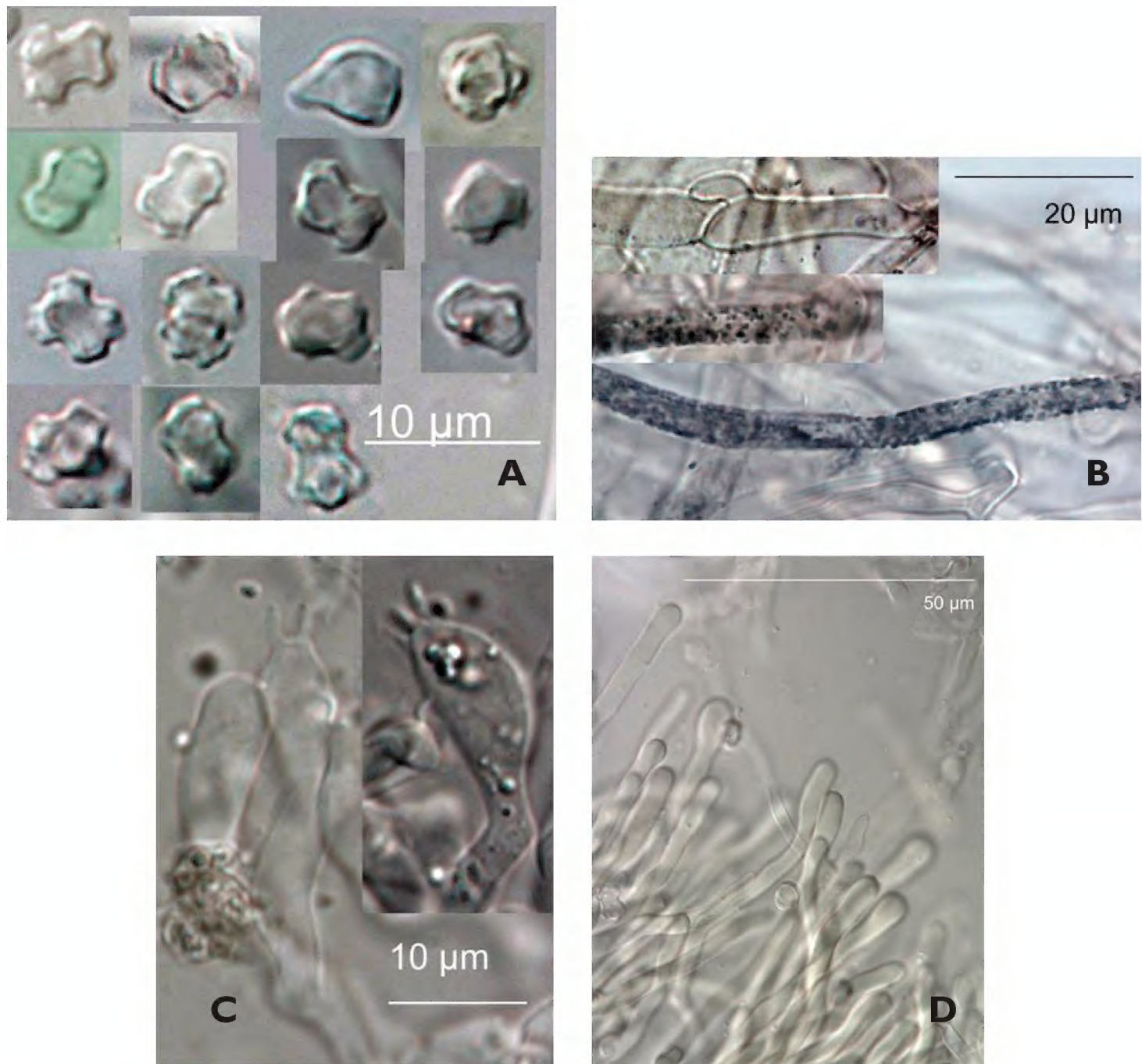


**Figure 2.** Basidiomes in natural habitat (PDD96007).



**Figure 3.** Basidiomes (PDD96007).





**Figure 4.** Microscopic Details. **A** Spores in Melzers (PDD96012) **B** Cap hyphae showing clamps and granules in KOH (PDD96012) **C** Basidia in KOH (PDD96012) **D** Cystidia-like elements in KOH (PDD96012)

**Etymology.** *nothofagi* for its ectomycorrhizal association with *Nothofagus*.

**Conservation status.** Although there are no data on the stability of the population size or historical changes in distribution of this species, it is likely to be naturally uncommon according to the New Zealand Threat Classification System (Townsend et al. 2008).

**Holotype.** NEW ZEALAND, North Island, Rimutaka Forest Park (under *Nothofagus fusca*), NZMG: 2671550E, 5982715N, 2<sup>nd</sup> May 201, D. Batchelor & P. Leonard, PDD96007 (PL3511)

**Other specimens examined.** New Zealand, North Island, Rimutaka Forest Park (under *Nothofagus fusca*), NZMG: 2671550E, 5982715N, 15<sup>th</sup> May 2009, T. Lebel, PDD95529 (JAC11078). West Bay, St Arnaud (*Nothofagus fusca*), South Island, NZMG: 1586280E, 5372097N, 11<sup>th</sup> May 2010, S. Kerr & P. Leonard, PDD96012.



**Discussion.** *Boletopsis nothofagi* differs from described species in the more elongate spores with a narrow central waist, granular extra-cellular material becoming green in KOH and habitat in *Nothofagus* forests (Niemala and Saarenoska 1989; Harrison 1975; Watling and Milne 2006).

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